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Expression of Adhesion Molecules in the Lymphoid Cell Distribution in Rheumatoid Synovial Membrane

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To investigate the mechanism of cellular infiltrates in the lining layer and lymphocytic infiltrates of the rheumatoid synovial membrane, immunohistochemical and immunoelectron microscopic studies utilizing monoclonal antibodies against the adhesion molecules, CD54 (ICAM-1), CD11a (LFA-1), and CDw49e (VLA-5) were carried out to determine the pattern of distribution of these molecules in the rheumatoid synovial membrane. Treatment with anti-ICAM-1 resulted in membrane staining of most of the lining cells, suggesting the possibility that ICAM-1 may function to facilitate the adhesion of ICAM-1 bearing type A cells to type B cells of the lining layer. ICAM-1 positive macrophages and fibroblasts were often in contact with lymphoid cells suggesting that cellular immune reaction occurs in the transitional area, and also that the lining cells may utilize VLA-5 for anchorage to fibronectin at the surface of the synovial membrane. ICAM-1 staining was weak in lymphoid aggregates, but strong in the transitional area, indicating a paucity of ICAM-1 bearing cells in the lymphocyte rich areas. On the other hand, LFA-1 staining was very strong in the lymphoid aggregates and only moderate in transitional areas. This suggests that the large numbers of T4 cells present in the lymphocyte rich areas are sufficiently activated to express substantial levels of LFA-1, and also that the LFA-1 molecule is an important receptor for emigration from postcapillary venules. The VLA-5 molecules stained in the transitional areas may provide appropriate adhesion and anchorage for the achievement of the variety of immune reactions which occur in these areas.

Key Words

Rheumatoid synovial membrane,
Adhesion molecules,
Lymphocyte distribution,
Lining layer.

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INTRODUCTION

Rheumatoid arthritis (RA) is characterized by chronic inflammation in synovial tissue. A prominent feature of RA is the chronic accumulation of leukocytes in the inflammed synovium. In the sublining region, the mononuclear cells are distributed in a variety of patterns[1,2]. In the previous studies using monoclonal antibody staining, it was found that lymphocyte rich areas are made up predominantly of CD4 cells, while the lymphocytes of the transitional areas were mainly CD8 cells[3-6]. In the process of

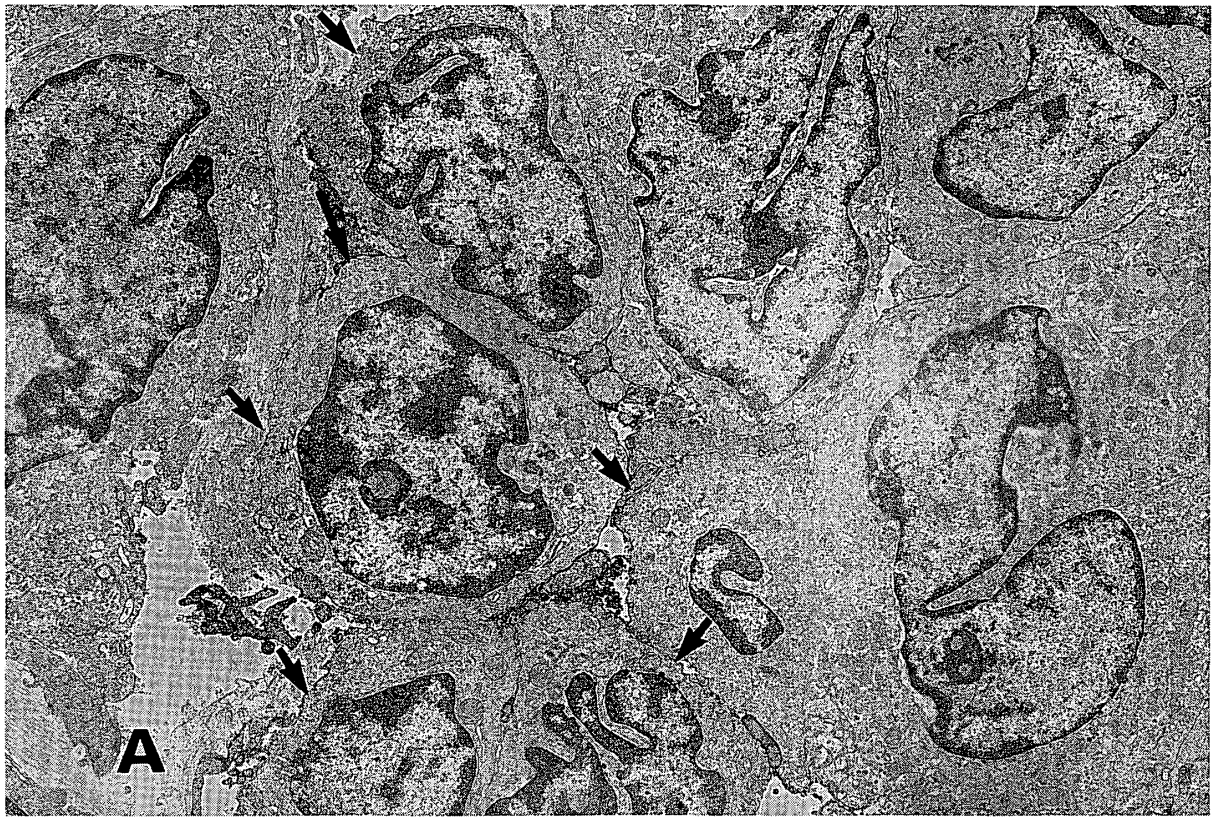
emigration, lymphocytes bind to endothelial cells (EC) of the postcapillary venules (PCV) by means of receptor-ligand interactions[7]. The endothelium at the PCV in lymphoid tissue is morphologically and functionally distinct. It is formed into high endothelial venules, which play a critical role in lymphocyte recirculation[4]. Cytokine-activated EC express increased levels of intercellular adhesion molecule-1 (ICAM-1)[8,9], endothelial leukocyte adhesion molecule-1 (ELAM-1)[10] and vascular cell adhesion molecule-1 (VCAM-1)[11] leading to increased binding of T cells. In addition, it has been observed that memory T cells express increased levels of the lymphocyte function associated antigen-1 (LFA-1)[12,13], very late activation antigen-4 (VLA-4)[14], and intercellular adhesion molecule-1 (ICAM-1)[15]. Such memory cells may congregate in chronic inflammatory infiltrates because 1) They emigrate in increased numbers as a result of interactions between the above receptor-ligand pairs and 2) they are retained extracellularly as a conpequence of interaction with other cells and with tissue matrix prteins[16–18]. *In the present study,* immunohistochemical staining has been carried out in order to further define the role of adhesion molecules in the homing of lymphocytes to the rheumatoid synovial membrane and their pattern in this tissue, using monoclonal antibodies against LFA-1, β-1 integrins and ICAM-1.

MATERIALS AND METHODS

Synovial tissues. Twenty eight synovial tissues from patients with rheumatoid arthritis were obtained at synovectomy or reconstructive joint surgery. All patients had classical seropositive disease and met the American College of Rheumatology criteria[19]. On gross examination, there was moderate to severe proliferation of the synovial membrane at the time of surgery in all cases.

Autisera and other reagents. Purified anti-human mouse monoclonal antibodies CD54 (ICAM-1), CDw49e (VLA-5) and CD11a (LFA-1) were purchased from Immunotech (Marseille Cedex, France). Avidin biotinylated peroxidase (ABC-Kit) was obtained from Vector Laboratories (Burlingame, Ca) and 3-3' diaminobenzidine from Sigma Chemical Co (St. Louis, Mo). Sections, 6νm thick, were cut on a cryostat (Bright Co. Huntingdon, England) at -20°C, and mounted on gelatin and egg albumin coated slides. After drying at room temperature, the section were washed with phosphate-buffered saline (PBS). Normal goat serum, diluted 1:200, was applied to the sections for 20 min. After washing, these were incubated with 100-200 ml of 1:50 diluted monoclonal antibody (anti-ICAM-1, anti-VLA-5, and anti-LFA-1) for 60 min. After washing with PBS, biotinylated peroxidase conjugated goat anti-mouse lgG antibody was added. The tissue was then incubated with 3 mg of 3-3' diaminobenzidine in 10 ml of 0.05M Tris HCl buffer, pH7.5, for 10 min. Specimens were then washed in PBS and dried at room temperature. As a control, specimens were treated with purified mouse lgG at a concentration of 10 μg/ml, and stained with hematoxylin and eosin. For electron microscopic

Fgiure 1. A-C, Adhesion molecules expressed in the lining layer and lymphocyte rich areas of rheumatoid synovial membrane. Sections stained for anti-LFA-1 (A), anti-ICAM-1 (B), and anti-VLA-5 (C). Note that LFA-1 staining was absent in the lining layer.



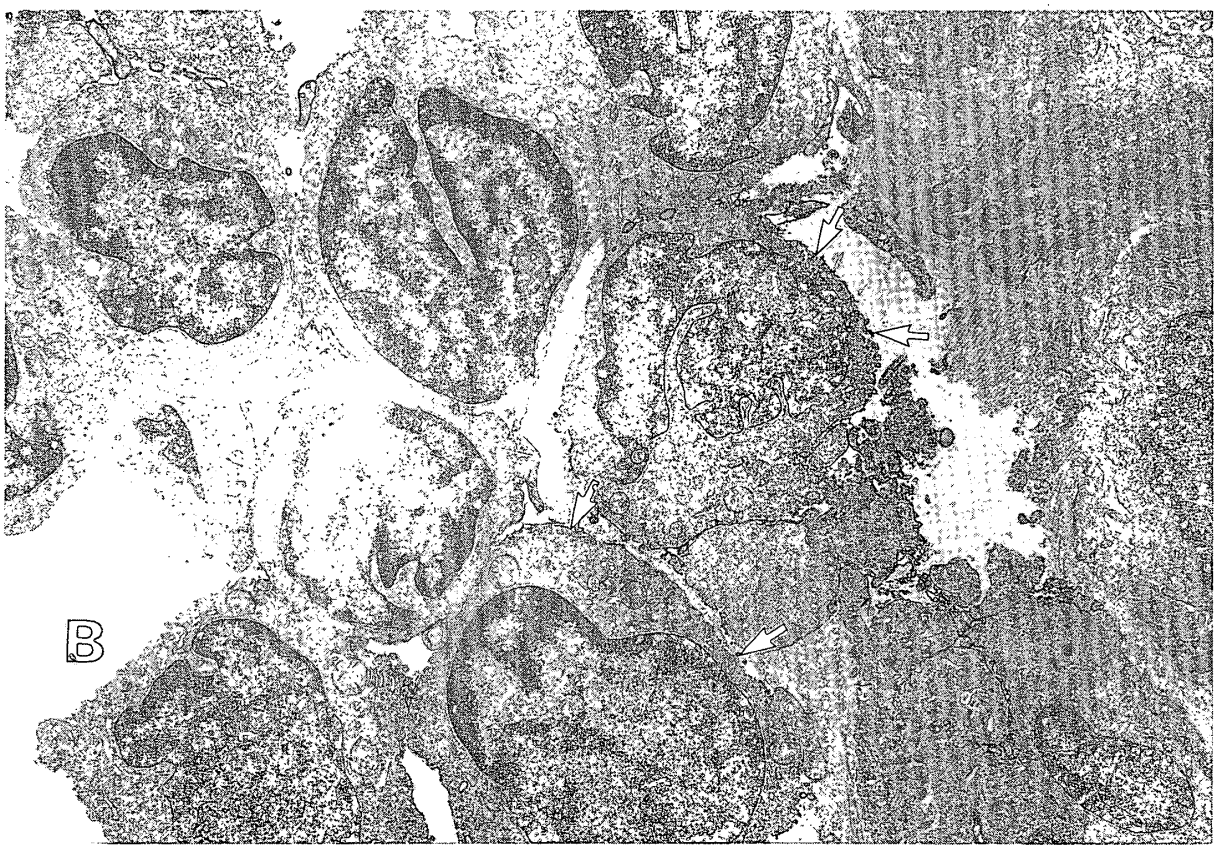
Fgiure 2. A. Part of the field in Fig. 1A, stained with LFA-1 antibody and observed without counterstaining in the electron microscope. Most of the lymphocytes are positive on membrane staining of LFA-1 antibody.

examination, the sections were fixed with 1% osmium tetroxide for one hour and washed in PBS, dehydrated in graded alchol to 100%. While the sections were still wet, plaptic capsules filled with Epon 812 were inverted over the sections. After polymerization of the Epon 812, the slides were heated on a hot plate and sections were removed from the slide. Sections were cut on an LKB microtome and examined in a Hitachi H-300 electron microscope.

RESULTS

When twenty eight RA synovial tissue samples were stained with anti-ICAM-1, anti-LFA-1 and anti-VLA-5, almost all cells of the lining layer showed strong ICAM-1 and VLA-5 staining. LFA-1 staining was absent in most of the specimens examined (Figures 1 A-C).

Lymphocyte-rich areas, which consist mostly of densely packed small lymphocytes[1,2], are made up mainly of T4 cells[3-5,20]. In such aggregates, most of the lymphocytes expressed LFA-1; however, a few were ICAM-positive. Only small numbers of VLA-5 positive cells were seen. When present, germinal center-like areas are usually located in the center of the lymphocyte-rich areas, and consist mostly of B lymphocytes[5,21]. In such areas, most of the lymphocytes expressed LFA-1^+, ICAM-1^+, and VLA-5^+. In figure 2A, A portion of the field stdined with LFA-1 antibody, viewed in figure 1A at the light microscopic level, is shown as it appears in the electron microscope without



Fgiure 2. B. Perivascular lymphocyte-rich collection stained with VLA-5 antibody. Membrane positive staining cells are occasionally seen (arrows).

counterstaining. It is seen that the cell membranes have been stained permitting positively staining cells to be identified by the specificity of their electron-dense rim staining as described by Kurosaka and Ziff[3]. When lymphocyte-rich areas were stained with LFA-1 antibody, the majority of the lymphocytes were found to be LFA-1 positive cells. When the lymphocyte-rich areas were stained with VLA-5 antibody, it was observed that the VLA-5 cells were much less frequent than the LFA-1 cells and were disributed in a scattered manner in the area stained (Fig. 2B).

At the edge of lymphocyte-rich areas, a transition usually occurs to areas which contain macrophages, lymphocytes and plasma cells[22]. The cells infiltrated in these areas are no longer aggregated and vary in size. Approximately half of the cells in these areas were LFA-1$^+$. In contrast to the lymphocyte rich areas, the transitional areas contained large numbers of ICAM-1$^+$ cells, and these cells appeared to be in contact with small lymphocytes. Also in marked contrast to the lymphocyte rich areas, where VLA-5 staining was absent, VLA-5$^+$ cells were present in large numbers in the transitional areas (Fig. 3 A, B, C). In transitional areas, the fraction of LFA-1 cells in the lymphocytes present was significantly reduced to about one-third of the total, while the fractions of VLA-5 and ICAM-1 cells rose, usually to exceed that of the LFA-1 cells (Fig. 4A, B). Not only was there a varity of cells in the transitional areas, but the cells were generally large in size and more loosely packed than in the lymphocyte-rich areas. These findings were very similar to those of Kurosaka's observation of OKT4 and OKT8[3].

As to the relation of LFA-1, ICAM-1 and VLA-5 cells to the percentage of lymphocytes in mononuclear infitrates, the above observation is further analyzed in Table 1 and Table 2. In these tables, mean values of the percentage of ICAM-1, LFA-1 and VLA-5 cells, expressed as percentage of total lymphocytes and macrophages are given. Thus lymphocyte rich areas was composed of lymphocytes of more than 60% of LFA-1 positive cells. It is seen that the LFA-1/ ICAM-1 and LFA-1/ VLA-5 ratios in the areas of lymphocytes-rich was about 2.0.

Macrophages which interact with lymphocytes, play an important part in the immune responses of these cells. These cells were seen in increased numbers in the transitional areas, and these macrophages expressed ICAM-1 and VLA-5 molecules in one third of cells.

DISCUSSION

To investigate the role of adhesion molecules in the mobilization and distribution of mononuclear cells in the rheumatoid synovial membrane, we studied the staining pattern of a group of these molecules in the hyperplastic lining layer, the lymphocyte-rich and transitional areas of the synovium. Focal hyperplasia of the synovial lining layer was seen in most patients with RA. Staining patterns of ICAM-1, LFA-1 and VLA-5 were somewhat different. The cells of the lining layer were strongly ICAM$^+$ and VLA-5$^+$ while LFA-1 staining was very

Table 1. Adhesion Molecule Expression on Lymphocytes in Lymphocyte-Rich and Transitional Areas

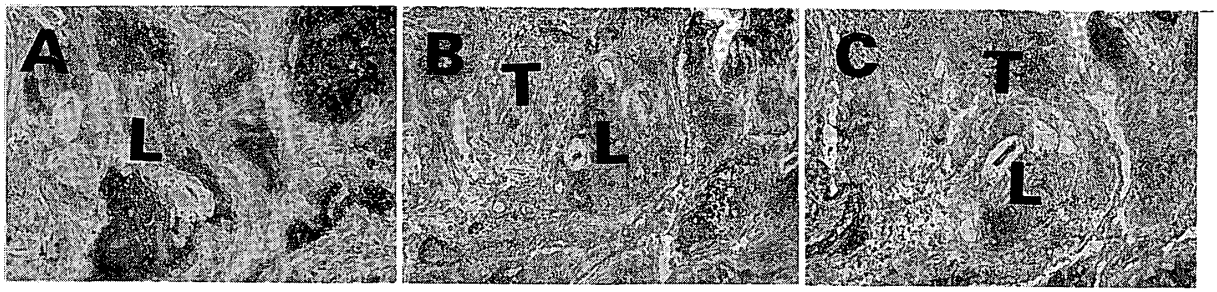
	Number of positive cells / Number of total cells counted		
	ICAM-1	LFA-1	VLA-5
Lymphocyte-rich area	40/123 (32.5%)	155/241 (64.1%) *	60/150 (40.0%)
Transitional area	32/116 (33.0%)	24/73 (33.0%) *	25/70 (35.7%)

(*$p<0.0005$)

Table 2. Adhesion Molecule Expression on Macrophages in Lymphocyte-Rich and Transitional Areas

	Number of positive cells / Number of total cells counted		
	ICAM-1	LFA-1	VLA-5
Lymphocyte-rich area	14/28 (50%)	2/9 (22.2%)	6/16 (37.5%)
Transitional area	32/53 (60.4%)	5/26 (15.4%)	14/28 (50.0%)

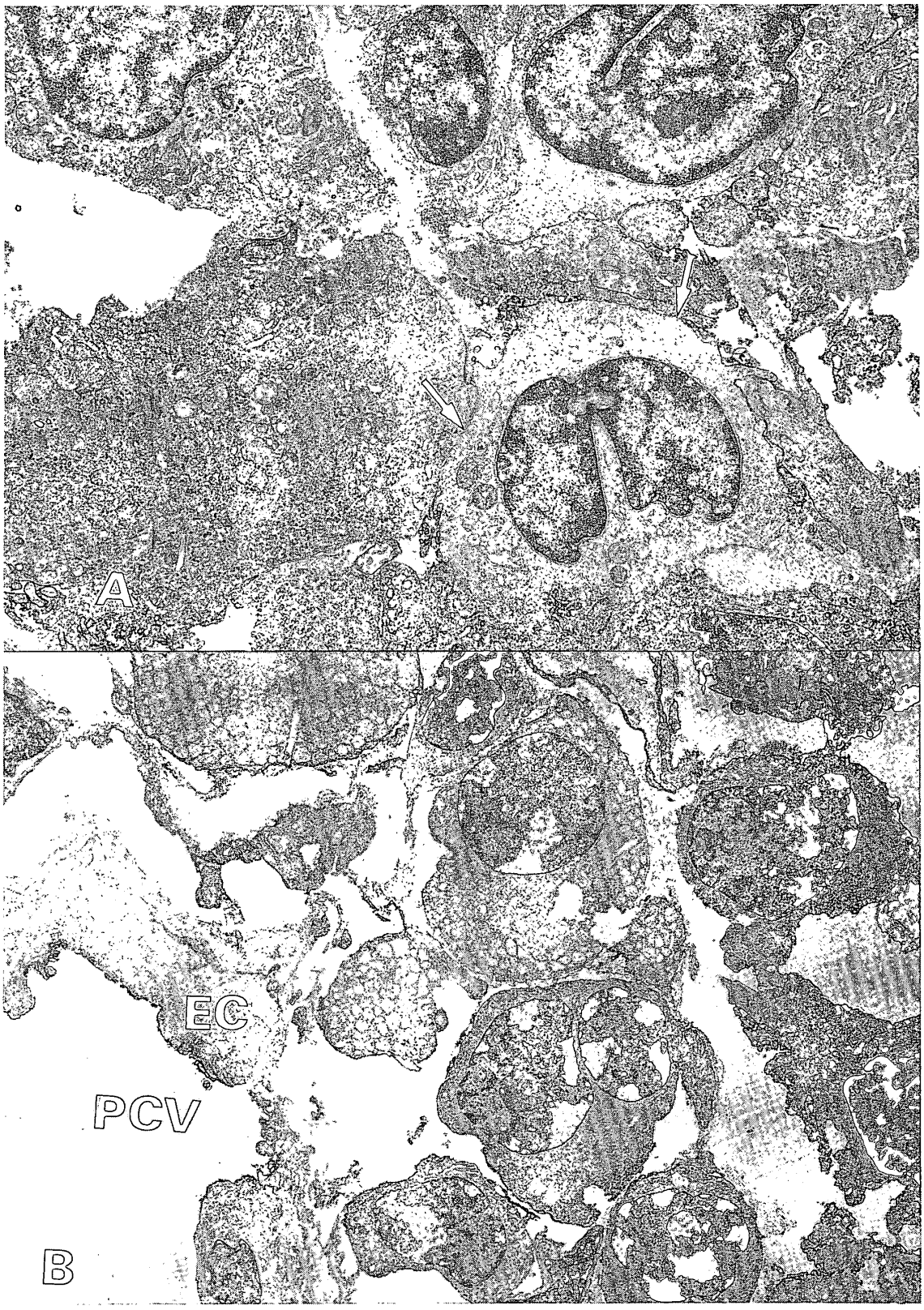
uncommon. Since lining cells have the characteristics of macrophages (type A cells) and fibroblastic cells (type B cells)[23], one may attribute the ICAM-1 staining to both of these cell types[24–27]. The VLA-5 staining may also be attributed to both the type A and type B cells in as much as macrophages[24–28] and such macrophages and fibroblant-like cells[29–31] have been shown to express VLA-5. These lining cells may also utilize VLA-5 for anchorage to fibronectin at the surface of the synovial membrane[32,33] for the development of the hyperplastic lining layer. VLA-5 may in addition facilitate the growth of the pannus by virtue of its ability to react with fibronectin secreted by the proliferating synoviocytes of the pannus[13,28,32,33]. It is likely that binding of lymphocytes, macrophages and fibroblasts in the synovial tissue, as observed in the present experiments, results from interaction between VLA receptors on



Fgiure 3. A-C, Transitional areas stained for anti-LFA-1 (A), anti-ICAM-1 (B), and anti-VLA-5 (C). L: lymphocyte rich area, and T: transitional area.

these cells and matrix protein ligands. Recent studies[16,17] have demonstrated interaction between adhesion molecules of the $\beta 1$ group (VLA-4 and VLA-5) of the integrin supergene family with fibronectin, collagen, and laminin of the connective tissue matrix, and stimulation of synoviocyte proliferation by reaction between membrane bound VLA-5 and fibronectin [11,30]. With regard to the increased expression of ICAM-1, the evidence that this ligand may interact with OKM-1 (αm, $\beta 2$) on the macrophage, as reported by Simmons et al[34], suggests the possibility that ICAM-1 may function to facilitate the adhesion of type A cells bearing OKM-1 to type B cells, and serve in this way to maintain the compact structure of the lining layer.

In the lymphocyte-rich areas, as previously reported by Koch et al[8], the EC of PCV were both ELAM-1^+ and ICAM-1^+. We have also observed similar staining levels for these molecules in the transitional areas. This is not unexpected since the lymphocytes, which consitute the bulk of the emigrating cells in the lymphocyte rich areas, and the monocytes, which, along with lymphocytes, emigrate in the transitional dreas, both react with ELAM-1[12,35–36] and ICAM-1[9,13,27,37]. However, the fact that lymphocytes emigrate tall endothelial PCV in lymphocyte rich areas in relatively large numbers com-



Fgiure 4. Perivascular transitional area stained with LFA-1 antibody (A), and ICAM-1 antibody (B). Note that the ICAM-1 is strongly expressed on the surface of endothelial cells of PCV.

pared to their emigration in transitional areas suggests that the tall EC of the PCV of lymphocyte rich areas express a ligand which is not well expressed by the flat EC of the transitional areas[38,39]. Thus, after an initial stimulus the adhesion molecules may play a role in the selective binding of inflammatory cells to endothelium and their subsequent migration to the synovial tissue as well as in the adhesion of these cells to both other cells and extracellular matrix proteins, resulting in accumulation and activation of inflammatory cells and perpetuation of synovitis[40].

Of interest was the very weak staining for ICAM-1 in the lymphoid aggregates and strong staining for this molecule in the transitional areas. This is best explained by the paucity of ICAM-1 bearing cells, such as macrophages and activated fibroblasts, in lymphoid aggregates, which consist mainly of T4 cells[3,5,20]. In contrast, the transitional areas are rich in macrophages and fibroblasts, which express ICAM-1[27,37]. In the present electron microscopic study, ICAM-1 positive macrophages and fibroblasts were often in contact with lymphoid cells. With regard to the increased expression of ICAM-1 on the cell present in the transitional area, the evidence that this ligand may interact with $\beta 2$ integrin on the macrophages, suggests the possibility that ICAM-1 may function to facilitate the adhesion of macrophages to lymphocytes.

Of interest also was the observation that LFA-1 staining was very strong in the lymphoid aggregates and only moderate in the transitional areas. This suggests that the large numbers of T4 cells present in the lymphocyte-rich areas are sufficiently activated to express substantial levels of LFA-1[12,13,36,41]. Electron microscopic examination of the varying areas of the synovium demonstrated that the percentage of LFA-1 cells in any given area was proportional to the percentage of lymphocytes in that area, with the lymphocyte-rich areas having the highest concentration of LFA-1 cells.

Also noteworthy was the fact that, in contrast to the strong staining of LFA-1 in lymphocyte rich areas, VLA-5 staining was very weak. This suggests that the LFA-1 molecule is an important receptor for emigration from tall endothelial PCV[13,37,41,42]. The moderately strong staining for both LFA-1 and VLA molecules in transitional areas raises the possibility that both of these molecules are involved in the emigration of lymphocytes from the flat endothelial cells which are characteristic of the PCV of transitional areas[38].

Rheumatoid synovial tissue is enriched for lymphocytes expressing high level of integrins[43–45]. Furthermore, in a previous study, we raised the possibility that VLA-5 may facilitate the growth of the synovium by virtue of its ability to react with fibronectin with resulting proliferating of the synovium[46]. Thus it is likely that the tissue distribution patterns of infitrated cells are influenced by ECM and the ability of cells to interact with the ECM through surface receptor expression. The presence in the transitional areas of increased numbers of macrophage-like cells in close contact with VLA-5 positive cells, is suggestive of the type of cellular interaction which occurs in autologous

mixed lymphocyte reactions[3,47].

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